D.M. KISCH INC., Johannesburg

Form P.1

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

APPLICATION FOR A PATENT AND ACKNOWLEDGEMENT OF RECEIPT
(Section 30 (1) - Regulation 22)
The grant of a patent is hereby requested by the undermentioned applicant on the basis of the present application filed in duplicate.

PATENT APPLICATION NO.		AGENT'S REFERENCE
21 01	995408	P/99/77882

FULL NAME(S) OF APPLICANT(S)	
71	UNIVERSITY OF PRETORIA OFFICE OF THE DIRECTOR, RESEARCH SUPPORT

REGISTRAR OF PATENTS, DESIGNS, TRADE MARKS AND COPYRIGHT
1999 -08- 24
REGISTRATEUR VAN PATENTE, MODELLE, HANDELSMERKE EN OUTEURSREG

ADDRESS(ES) OF APPLICANT(S)	
	Pretoria 0001

TITLE OF INVENTION			
54	A BIOREACTOR		
X	THE APPLICANT CLAIMS PRIORITY AS SET OUT ON THE ACCOMPANYING FORM P.2. The earliest priority claimed is ZA No. 98/4619 dated 29-05-1998		
	THIS APPLICATION IS FOR A PATENT OF ADDITION TO PATENT APPLICATION NO.	21	01
	THIS APPLICATION IS A FRESH APPLICATION IN TERMS OF SECTION 37 AND BASED ON APPLICATION NO.	21	01

THIS APPLICATION IS ACCOMPANIED BY :

X	1	~~A single copy of a complete specification~~ two copies of a complete specification of 18 pages.	
X	2	Drawings of 1 sheets.	
X	3	Publication particulars and abstract (Form P.8. in duplicate).	
X	4	A copy of Figure 3 of the drawings for the abstract.	
X	5	An assignment of invention.	
	6	Certified priority document(s) (State number).	
	7	Translation of priority document(s).	
	8	An assignment of priority rights.	
X	9	A copy of Form P.2 and specification of S.A. Patent Application No.	21 01 98/4619
X	10	A declaration and power of attorney on Form P.3.	
	11	Request for ante-dating on Form P.4.	
	12	Request for classification on Form P.9.	
X	13	Extension of 3 months on Form P4	

DATED THIS 24 th DAY OF August 19 99

Patent Attorney for the Applicant(s)

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Form P.7

REPUBLIC OF SOUTH AFRICA

PATENTS ACT, 1978.

COMPLETE SPECIFICATION

(Section 30 (1) - Regulation 28)

PATENT APPLICATION NO.			LODGING DATE.		AGENT'S REFERENCE
21	01	995408	22/23	24-08-1999	P/99/77882

INTERNATIONAL CLASSIFICATION	
51	C12N B65 D

FULL NAME(S) OF APPLICANT(S)	
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TITLE OF INVENTION	
54	A BIOREACTOR

INTRODUCTION AND BACKGROUND TO THE INVENTION

This invention relates to a method and bioreactor for cultivating microorganisms.

A bioreactor which is conventionally used for relatively small - scale anaerobic bioreactions is known as the GasPak™ jar. The GasPak jar comprises a sealed glass container of which the inside is rendered anaerobic by the removal of oxygen through the formation of water by a chemical reaction using hydrogen and a palladium catalyst. Another known anaerobic bioreactor comprises a glass bottle which is sealed by a closure cap. The inside of such bottle is made anaerobic by using, for example, reducing agents, vacuum pumps and flushing agents, to remove oxygen from the inside of the bottle.

A common disadvantage of the known bioreactors is that they are too expensive for use where a plurality of relatively small - scale bioreactions are required. The mere use of such bioreactors is also relatively expensive due to the cost of the catalysts, reducing agents, and flushing agents that are required when using these known bioreactors.

OBJECT OF THE INVENTION

It is accordingly an object of the present invention to provide a method and bioreactor for cultivating microorganisms with which the aforesaid disadvantages may be overcome or at least minimised.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided a bioreactor comprising a disposable, extensile, sealed pouch.

Preferably the pouch is transparent.

The pouch may be provided with an inlet which is closed by a resilient seal which may be removable or alternatively which may be pierceable by a needle of a syringe or the like, for the introduction of matter into the pouch.

The pouch may optionally include a vent for the release of pressurised fluid which may be formed during a bioreaction taking place inside the pouch.

The pouch may contain a suitable medium for supporting a bioreaction.

The medium may further be inoculated with a suitable microorganism for performing such bioreaction.

Preferably the pouch is portable.

According to a second aspect of the invention there is provided a bioreactor suitable for use in a relatively small - scale bioreaction, the bioreactor comprising a portable, sealable, extensile pouch.

The pouch may be disposable.

According to a third aspect of the invention there is provided a bioreactor comprising an infusion bag - type pouch.

According to a fourth aspect of the invention there is provided a method for cultivating microorganisms including the steps of:

- providing an infusion bag - type pouch;
- introducing a suitable medium for supporting a bioreaction into the pouch; and
- inoculating the medium with a suitable microorganism.

According to a fifth aspect of the invention there is provided a method for cultivating anaerobic microorganisms including the steps of:

- providing a disposable, extensile, sealed pouch;

- introducing a suitable medium for supporting a bioreaction into the pouch; and
- inoculating the medium with an anaerobic microorganism.

Further according to the invention, the inside of the pouch is free of oxygen prior to the step of introducing a suitable medium into the pouch.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described further, by way of example only, with reference to the accompanying drawings wherein:

figure 1 is a plan view of a bioreactor according to a preferred embodiment of the invention prior to the introduction of a suitable medium for supporting a bioreaction into the bioreactor;

figure 2 is the same view as that of figure 1, showing the introduction of such medium into the bioreactor; and

figure 3 is also a plan view of the bioreactor of figure 1, after a bioreaction has taken place inside the bioreactor.

DESCRIPTION OF A PREFERRED EMBODIMENT OF THE INVENTION

Referring to figure 1, a bioreactor according to a preferred embodiment of the invention, is generally designated by reference numeral 10.

The bioreactor 10 comprises a portable, disposable, transparent, extensile pouch 12 having an inlet 14 which is sealed by a seal 16. Referring to figure 2, the seal 16 is pierceable by a needle of a syringe or the like to introduce a medium into the pouch 12, as described below.

Alternatively, the seal 16 may be in the form of a removable plug which facilitates the introduction of a medium having a relatively high viscosity or thick constitution into the pouch 12. Referring again to figure 1, the construction of the bioreactor 10 is preferably similar to or the same as that of an infusion bag.

The pouch 12 is tubular with opposed ends of the tube being closed by heat sealing. The walls of the pouch 12 includes an inner layer of polyvinyl chloride and an outer layer of high-density polyethelene. The seal 16 is preferably made from latex and during manufacturing of the bioreactor 10, care is taken to ensure that the inside of the pouch 12 is oxygen free and sterile.

In use, a suitable medium 18 (described in more detail below) for supporting a bioreaction, is introduced to the inside of the pouch 12 as shown in figure 2. The medium 18 is preferably inoculated with a suitable microorganism for performing a bioreaction, prior to the step of introducing the medium 18 into the pouch 12. It will however be appreciated that a plurality of bioreactors 10 according to the invention may first be prepared by the introduction of media 18 and with different microorganisms being inoculated into the medium 18 in each pouch 12 respectively, at a later stage. For example, when samples are taken during experimentation, each pouch 12 may be inoculated with an isolate of a different anaerobic microorganism.

A bioreaction is allowed to take place inside the bioreactor 10. Referring to figure 3, as described in more detail below, during the bioreaction, gas is usually formed inside the pouch 12 and which increases the pressure inside the bioreactor 10. This increased pressure inflates the pouch 12 and in order to prevent the pouch 12 from bursting, the bioreactor 10 is further provided with an optional safety vent 22 in the form of a one-way valve.

Specific examples of the use of the bioreactor 10 will now be described below to illustrate the effectiveness and suitability of the bioreactor 10 according to the invention for use in relatively small - scale bioreactions, where a plurality of bioreactions are required to take place simultaneously.

according to the invention for use in relatively small - scale bioreactions, where a plurality of bioreactions are required to take place simultaneously.

Media composition

Table 1. Artificial acid mine drainage (AMD) composition.

Component	Quantity
$MgSO_4.7H_4O$ (Merck)	1.31g
H_2SO_4 (96%) (Merck)	0.30ml
$FeSO_4.7H_2O$ (Saarchem)	4.56g
NH_4Cl (Labchem)	0.19g
H_2PO_4 (85%) (Merck)	0.02ml

The above chemicals (Table 1) were dissolved in one litre of distilled water. The pH was adjusted to 7.2 using 10M Sodium hydroxide (Merck). By using this formula, theoretically, an average sulphate concentration of 2 500mg/L should be achieved. The SQ118 spectroquant (Merck) (kit no. 1.14791.00010) was used to determine the sulphate concentration of the AMD.

Standardisation of the inoculum

before digester sludge, obtained from the Daspoort Water Purification Plant in Pretoria, South Africa, was added. Volumes of 100ml, 200ml and 300ml of inoculum were added to the AMD and lactic acid mixtures, respectively.

Chemical analysis

A SQ118 spectroquant (Merck) was used to determine the amount of sulphate reduction using kit no. 1.14791.0001.

Preliminary sulphate reduction tests on AMD

A volume of 300ml inoculum was added to 1000ml of artificial AMD (Table 1). A carbon source was also added to this mixture (Table 2). Of this, 900ml was introduced into a plurality of bioreactors 10 according to the invention respectively. The pH was adjusted to 7.1-7.5.

Table 2. Defined carbon sources used during this experiment

Carbon sources	Quantity per 100ml AMD
Lactic acid (92%) (Merck)	5.56ml
Acetic acid (Merck)	5.95ml
Butyric acid (BDH)	4.80ml

All experiments were done in triplicate. The sulphate concentration of the different bioreactors 10 were monitored every 2-3 days. As a control experiment, only AMD and inoculum was used with no added carbon sources.

3.3 Results and Discussion

Media composition

The average sulphate concentration of the artificial AMD was 2356mg/l. This resembles the sulphate concentration of AMD.

Standardisation of the inoculum

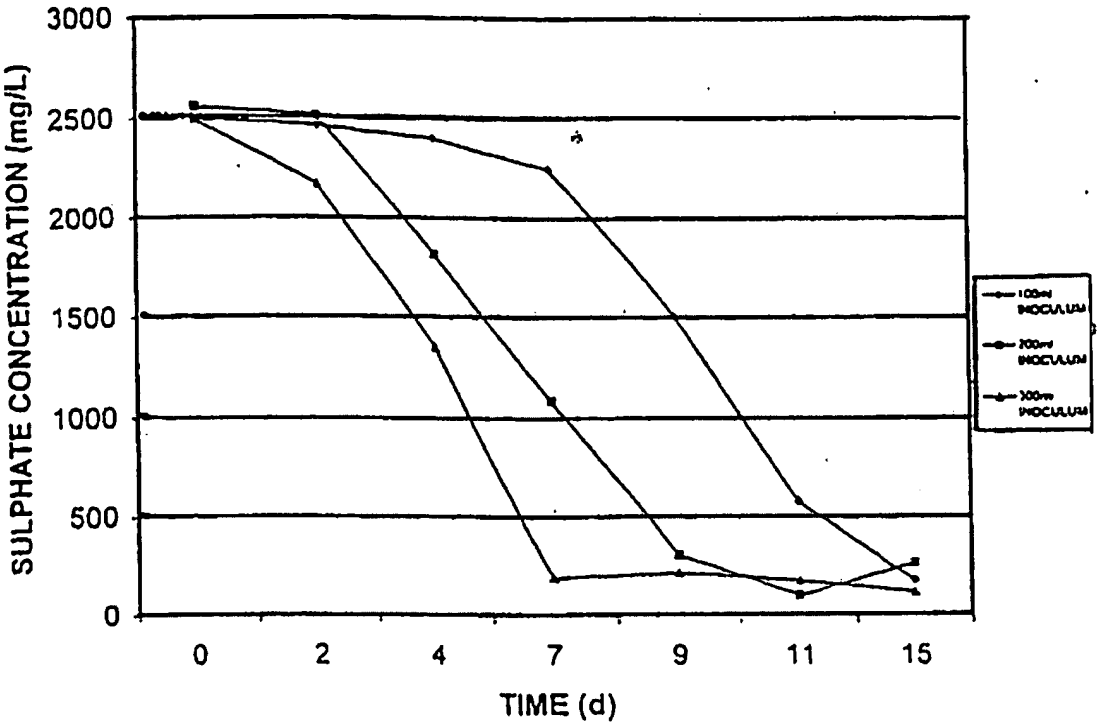

Diagram 1. Sulphate reduction using 100ml, 200ml, and 300ml of inoculum respectively.

Digester sludge consists mostly of fermentative bacteria and have low numbers of SRB (Zhender, 1988). The 300ml inoculum proved to be the most suitable concentration of inoculum (Diagram 1). Effective sulphate reduction was also obtained using 300ml inoculum (Diagram 1).

Preliminary sulphate reduction tests with AMD

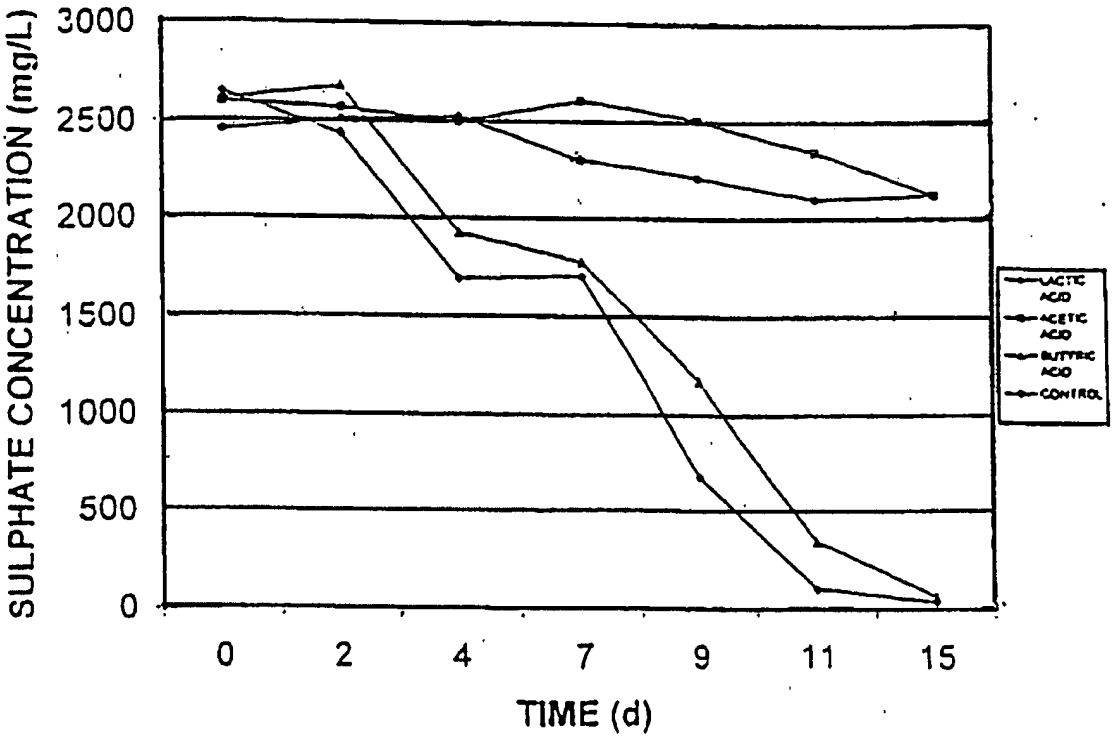

Diagram 2. Preliminary sulphate reduction tests using lactic acid, acetic acid and butyric acid as carbon sources.

Preliminary sulphate reduction tests with AMD were done to determine whether the bioreactor 10 was suited to be used as an anaerobic bioreactor. It was found that during a two week period during which experimentation took place, lactic acid and butyric acid proved to be good carbon sources for the removal of sulphate while acetic acid was not efficient (Diagram 2). These

results confirmed studies done by other researchers (Isa *et al.*, 1986; Ahring and Westerman, 1987; Joubert, 1987; Zhender, 1988; Qatibi *et al.*, 1990; Visser *et al.*, 1993).

In view of the above results, the bioreactor 10 according to the invention proved to be effective, inexpensive, and thus suitable for use when a plurality of relatively small-scale anaerobic bioreactions are required. During all of the sulphate reduction tests, gas was produced . The bioreactors 10 remained sealed and no gas leakage occurred. This was illustrated by the fact that the bioreactors 10 inflated to their maximum extensile capacity as shown in figure 3. The seal 16 provided a convenient sampling port through which gas could be removed by syringes or the like.

Furthermore, the bioreactor 10 did not require the addition of catalysts to achieve anaerobic conditions and no contamination of oxygen occurred.

It will be appreciated that a large number of variations in detail are possible with a method and bioreactor according to the invention without departing from the scope and/or spirit of the appended claims.

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Qatibi,A.I., Bories, A., Garcia, J.L. 1990. Effects of sulfate on lactate and C2-C3- volatile fatty acid anaerobic degradation by a mixed microbial culture. Antonie van Leeuwenhoek,**58:** 241-248.

Visser,A., Beeksma, I., van der Zee, F., Stams, A.J.M., Lettinga, G. 1993. Applied Microbiology and Biotechnology, **40:** 540-556.

Zehnder, A.J.B. 1988. Biology of Anaerobic Microorganisms. John Wiley and Sons Publishers. New York

CLAIMS

1. A bioreactor comprising a disposable, extensile, sealed pouch.

2. A bioreactor according to claim 1 wherein the pouch is transparent.

3. A bioreactor according to claim 1 or claim 2 wherein the pouch is provided with an inlet which is closed by a removable resilient seal.

4. A bioreactor according to claim 1 or claim 2 wherein the pouch is provided with an inlet which is closed by a seal which is pierceable by a needle of a syringe or the like, for the introduction of matter into the pouch.

5. A bioreactor according to any one of the preceding claims wherein the pouch includes a vent for the release of pressurised fluid which is formed during a bioreaction taking place inside the pouch.

6. A bioreactor according to any one of the preceding claims wherein the pouch contains a suitable medium for supporting a bioreaction.

7. A bioreactor according to claim 6 wherein the medium is inoculated with a suitable microorganism for performing such bioreaction.

8. A bioreactor according to any one of the preceding claims wherein the pouch is portable.

9. A bioreactor suitable for use in a relatively small - scale bioreaction, comprising a portable, sealable, extensile pouch.

10. A bioreactor according to claim 9 wherein the pouch is disposable.

11. A bioreactor comprising an infusion bag - type pouch.

12. A bioreactor substantially as herein described and as illustrated in the accompanying drawings.

13. A method for cultivating microorganisms including the steps of:

- providing an infusion bag - type pouch;
- introducing a suitable medium for supporting a bioreaction into the pouch; and
- inoculating the medium with a suitable microorganism.

14. A method for cultivating anaerobic microorganisms including the steps of:

- providing a disposable, extensile, sealed pouch;
- introducing a suitable medium for supporting a bioreaction into the pouch; and
- inoculating the medium with an anaerobic microorganism.

15. A method according to claim 14 wherein the inside of the pouch is free of oxygen prior to the step of introducing a suitable medium into the pouch.

16. A method for cultivating microorganisms substantially as herein described with reference to the accompanying drawings.

DATED AT PRETORIA THIS 24th DAY OF AUGUST 1999.

D M KISCH INC

PATENT ATTORNEYS FOR THE APPLICANT

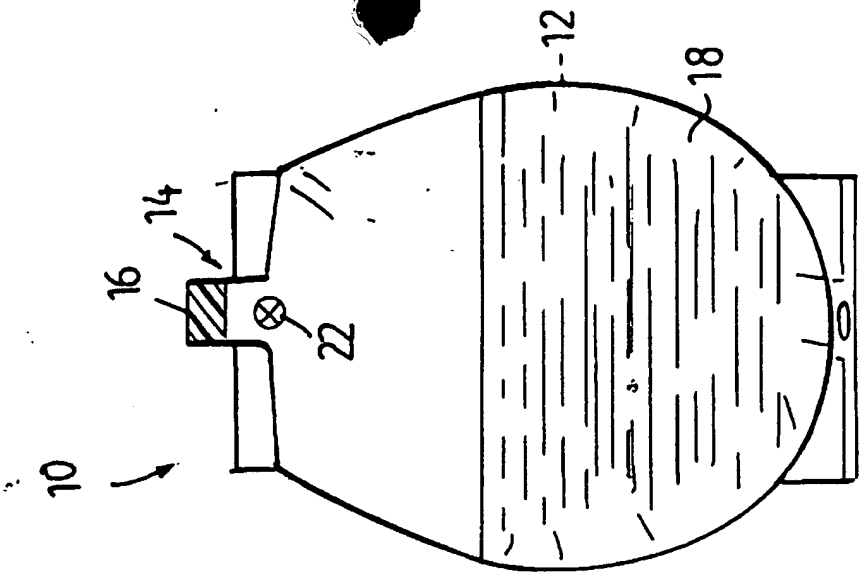

FIGURE 3

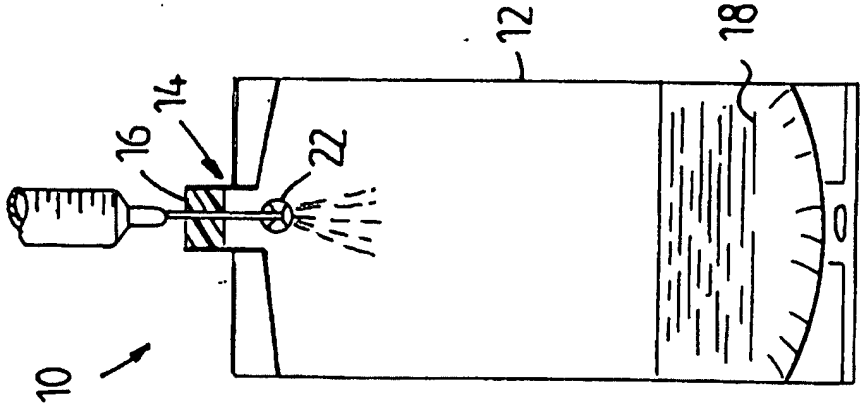

FIGURE 2

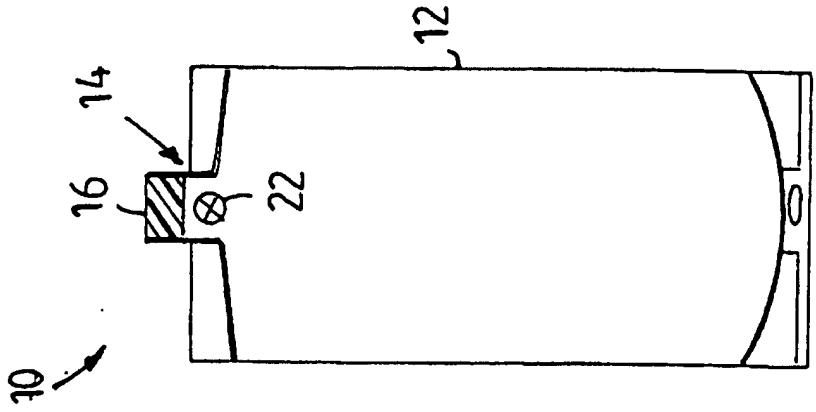

FIGURE 1

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Patent Attorneys for the Applicant